3 YIELD GEL FOR ASSESSING QUALITY AND QUANTITY OF ISOLATED DNA

3.1 TECHNICAL NOTES

3.1.1 Yield gels are best interpreted from the photograph because of excess fluorescence and background from the transilluminator. This also reduces the danger of overexposure to UV light.

3.1.2 Results serve as a quantitative tool for estimating the amount of DNA as well as a qualitative indication of sample integrity.

3.1.3 Non-human DNA present in a sample may produce results on the gel. These results may be indistinguishable from human DNA results.

3.1.4 Quantitation standards contain K562 of known concentrations. A range of standards between 15 and 500 ng is generally used.

3.1.5 Ethidium bromide (EtBr) is used to detect DNA by staining the yield gel. The ethidium bromide intercalates into the DNA molecule and fluoresces under UV light.

3.1.6 A UV transilluminator, at a wavelength of 302 nm, is used to visualize the fluorescent reaction between the EtBr and the DNA. At this wavelength, the saturation point of the DNA is 250-500 ng with a lower threshold of 5 ng. Therefore, samples exceeding this range should be diluted and requantitated.

3.1.7 The visual marker, used to monitor migration, contains known concentrations of lambda DNA digested with the restriction enzyme Hind III. Eight bands with the following molecular weights are present in this digest:

23,130 base pairs (bp)
9,416 bp
6,557 bp
4,361 bp
2,322 bp
2,027 bp
564 bp (may not be detected)
125 bp (not usually detected)

3.2 EQUIPMENT

3.2.1 Pipettes - 10 µL and 20 µL

3.2.2 Microcentrifuge

3.2.3 Gel tank, cover, and electrodes

3.2.4 Power supply

3.2.5 Heat block, 56° C

3.2.6 Vortex mixer

3.2.7 Microcentrifuge tube rack

3.2.8 Voltmeter

3.3 MATERIALS

3.3.1 Agarose gel, 16 or 20 cm (Refer to Appendix C.)

3.3.2 Microtiter plate

3.3.3 Sterile ART tips for pipettes - 10 µL and 20 µL

3.3.4 Gloves

3.4 REAGENTS

3.4.1 5X Loading buffer

3.4.2 Quantitation standards (K562)

3.4.3 Visual marker - Lambda Hind III (Use is optional.)

3.4.4 Ethidium bromide - 5 mg/mL (Optional - refer to Appendix C.)

3.4.5 1X TAE buffer

3.5 PROCEDURE

NOTE: The yield gel procedure for assessing the quality and quantity of isolated DNA should not be used for casework samples in place of the AluQuant® Human Quantitation procedure for assessing the quantity of isolated DNA addressed in Section 4. However, the yield gel procedure may be used to evaluate and dilute casework samples believed to contain a high concentration of DNA prior to the AluQuant® Human Quantitation procedure and to quantitate known reference samples.

3.5.1 Following the procedure outlined in Appendix C, prepare a 1% yield gel using DNA typing grade agarose in 1X TAE buffer.

3.5.2 Remove the quantitation standards and the visual marker from the freezer and bring to room temperature (or thaw at 56° C for 5 minutes). Vortex the tubes and then microcentrifuge briefly to mix the contents.

An example of suggested quantitation standards follows:

500 ng/6 µL
250 ng/6 µL
125 ng/6 µL
63 ng/6 µL
31 ng/6 µL
15 ng/6 µL

3.5.3 After the DNA has resolubilized, spin the tube for 5 seconds in a microcentrifuge to remove any condensation that may be on the lid.

3.5.4 Add 2 µL of 5X loading buffer to the appropriate number of wells of a microtiter plate.

3.5.5 Aliquot 4 µL of each sample into a different well in the microtiter plate.

3.5.6 Load the yield gel into the tank and add sufficient 1X TAE Buffer to cover the gel.

3.5.7 Load 6 µL of visual marker into the first well of the gel. When loading the wells, take great care that DNA does not trail up out of the wells. Make sure there are no air bubbles at the end of the pipette tip. Remove the pipette slowly and carefully after gently voiding.

3.5.8 Load the quantitation standards in order of <u>decreasing</u> concentration into the yield gel, such as:

2nd well	6 µL 500 ng control
3rd well	6 µL 250 ng control
4th well	6 µL 125 ng control

... and so forth until 6 µL of each quantitation standard has been loaded in wells 2 through 7 of the yield gel.

3.5.9 Load samples from the microtiter plate into the remaining wells of the yield gel.

3.5.10 Place the cover on the gel tank so that the red (positive) electrode is <u>farthest</u> from the loading wells.

3.5.11 Plug the red (positive) electrode into the positive plug of the power supply. Plug the black (negative) electrode into the negative plug of the power supply.

3.5.12 Turn the power supply on and set the voltage to the equivalent of 200 volts (power supply reading). Electrophorese for 30 minutes or until the loading buffer moves 2-3 cm from the origin.

NOTE: If ethidium bromide was not incorporated into the yield gel, the gel must be stained with ethidium bromide after electrophoresis. Soak the gel in 200 mL of 1X TAE and 40 µL of EtBr for 20-30 minutes.

WARNING!

ETHIDIUM BROMIDE IS MUTAGENIC. ALWAYS WEAR GLOVES AND A LABORATORY COAT WHEN HANDLING EtBr. IF USING EtBr SOLUTION TO STAIN THE GEL AFTER ELECTROPHORESIS, NEUTRALIZE THE SOLUTION WITH DOWEX 2 BEADS BEFORE DISCARDING.

3.5.13 View the yield gel on a UV transilluminator or in a UV viewing cabinet. Following the steps outlined in Appendix D, Steps 3.1 or 3.2, as appropriate, photograph the gel.

3.5.14 Determine the concentration of the DNA sample by comparing its intensity to the quantitation standards (K562). Divide the concentration by 4 µL, the volume of the sample added to the yield gel. This calculation will give the DNA concentration in ng/µL.

3.5.15 If the extracted DNA is below the detection level, and therefore cannot be visualized on the yield gel, the sample will be carried through the amplification process.

3.5.16 Continue with Section 5, PCR Amplification.

♦**END**